Effects of Inoculum Concentration and Host Age on Infection of Geranium by *Botrytis cinerea*

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ABSTRACT

Sirjusingh, C., Sutton, J. C., and Tsujita, M. J. 1996. Effects of inoculum concentration and host age on infection of geranium by *Botrytis cinerea*. Plant Dis. 80:154-159.

Conidial concentration and age of host organs were investigated in relation to infection of geranium flowers and leaves by *Botrytis cinerea*. Infection was assessed indirectly by estimating sporulation incidence of the pathogen in inoculated tissues. Threshold concentrations of conidia for inferred infection were ≤10 spores per ml for petals, 10 to 100 spores per ml for sepals, stamens, pistils, and pedicels, and 10^3 to 10^4 spores per ml for 4- to 6-week-old leaves. Infection efficiency in the leaves increased more than 200-fold when geranium petals were inoculated with conidia and positioned on leaves, compared with direct conidial inoculation. A linear regression model adequately described sporulation incidence and, by inference, infection incidence of sepals, stamens, pistils, and pedicels as a function of $\log_{10}$ conidial concentration. Logistic regression models closely described these relationships in petals and leaves. Sporulation incidence in leaves inoculated with conidia or with petals treated with conidia was high in 1-week-old leaves, declined as leaf age increased to 4 weeks, and increased as leaf age increased from 4 to 10 weeks. Observations have applications in the epidemiology and management of gray mold.

Gray mold, or Botrytis blight, of geranium (*Pelargonium* × *hortorum* L. H. Bailey), caused by *Botrytis cinerea* Pers.: Fr., is ubiquitous and destructive in geranium production systems in greenhouses in North America (11). Chief symptoms of the disease are flower blight, leaf blight, and stem rot (11,27). Management of gray mold depends heavily on fungicides; this is a matter of concern because of high costs, occupational exposure of greenhouse workers to fungicides, residues of fungicides on the crop and in the environment, and development of fungicide resistance in populations of *B. cinerea* in greenhouses (1,6,19,23,24). Control methods and strategies that require minimal amounts of fungicide could probably be developed based on quantitative epidemiologic information, but this is generally sparse. Infection, used here to mean the process of establishment of a parasite in the host (7), is an important epidemiologic component that is poorly understood.

Aerially dispersed conidia and mycelium in fallen petals of geranium are the chief forms of inoculum of *B. cinerea* in geranium production systems (11,12,14). Melchers (21,22) found that conidia of the pathogen can infect the flowers directly and produce symptoms on the petals and pedicels. Investigators have considered that conidia can also infect nonwounded and noninjured leaves (11,22), a possibility supported by observations that conidia, in the presence of 1% sucrose plus 0.25% peptone or 0.1 M dextrose, apparently infected geranium leaf disks (17,23). However, the importance of direct infection of the leaves by conidia under conditions of geranium cropping systems remains to be demonstrated. Leaves are readily infected by mycelium of *B. cinerea* in fallen petals of geranium (22). Fresh wounds on geranium stems are commonly infected by conidia of *B. cinerea* (13). Age of the host organ is an important variable influencing infection of several hosts by the pathogen (4,16,18), but has not been explored in geranium.

In the present study, inoculum concentration of *B. cinerea* and age of host organs were examined in relation to infection of the leaves and flower parts of geranium. Conidia were used to inoculate flowers and leaves, and petals treated with various concentrations of conidia were used as inoculum on leaves.

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Accepted for publication 16 November 1995.

Publication no. D-1995-1212-04R

MATERIALS AND METHODS

Host plants. Plants of geranium cv. Americana White (Valk Greenhouses Ltd., Grimsby, ON), a white-flowered tetraploid, were produced from terminal shoot cuttings that were rooted for 7 to 10 days in a greenhouse propagation bed and subsequently grown in 10-cm-diameter pots containing a soilless potting mix (Pro-mix, Plant Products Co. Ltd., Brampton, ON) in a climate-controlled greenhouse. Each plant was supplied twice weekly with 160 ml of soluble fertilizer (20-8-20 NPK plus micronutrients, Plant Products Co. Ltd.; 5 g per liter of water). During the day (0800 to 2000 hours) and at night (2000 to 0800 hours), air temperature averaged 18 to 24°C and 13 to 17°C, respectively, and relative humidity (RH) was 40 to 60% and 60 to 80%, respectively, with periodic nighttime peaks of 85 to 95%. White shades in the greenhouse roof were retracted when irradiance was <800 μE s^{-1} m^{-2} and drawn when irradiance exceeded 1,200 μE s^{-1} m^{-2} and at night to reduce heat loss. The plants were used for inoculations at 5 to 10 weeks after transplanting.

Inoculum and inoculations. Isolate GR-1 of *B. cinerea* from a fungicide-free geranium grown near Guelph was used for all inoculations. Conidia of the pathogen were produced on potato-dextrose agar (PDA) under cool-white fluorescent lamps (24-h photoperiod) at 20 to 22°C, recovered in sterile distilled water plus surfactant (0.1 ml Triton X-100/100 ml), shaken vigorously, filtered through two layers of sterilized cheesecloth, counted with the aid of a hemacytometer, and diluted to desired concentrations in water plus the surfactant. Percent germination of conidia was estimated on PDA and consistently exceeded 96% after 24 h at 21°C.

Conidial suspensions and petals inoculated with conidia were used as inoculum. Conidial suspensions were applied to incipient runoff on all surfaces of attached leaves and flowers of geranium using a 200-ml capacity air-pressurized hand sprayer that delivered a fine mist. To produce petal inoculum, attached flowers were sprayed 4 to 5 days after opening with *B. cinerea* (10^5 conidia per ml of sterile distilled water plus surfactant unless otherwise specified) or with the water plus surfactant only, and kept in high humidity at 20 to 25°C for 24 h. For inoculation treated petals were unfolded and positioned on adaxial surfaces of leaves to which they adhered with aid of residual moisture. Plants were inoculated with conidia or with petal inoculum at 0900 hours, and immediately placed in a clear plastic humidity chamber (1.5 m long × 0.5 m wide × 0.5 m high) in a greenhouse for 24 h. An ultrasonic humidifier was operated continuously in the chamber to maintain RH at or near saturation. A lid on the chamber was raised about 5 mm to allow exchange of air and water vapor. Chamber RH and air temperature were monitored with a calibrated hygrothermo-

graph. The temperature ranged from 22 to 27°C (day) and from 16 to 20°C (night). After the postinoculation humid period plants were kept on a bench in the greenhouse for 24 h, then assessed for infection incidence.

Estimation of infection incidence. Infection was assessed indirectly by estimating sporulation incidence of *B. cinerea* in flowers that were detached from inoculated inflorescences and in 1-cm-diameter leaf disks. The disks were cut from arbitrary sites on leaves inoculated with conidia and from sites at which petal inoculum was placed. Sites of petal adhesion were marked and petals were removed immediately before disks were cut from the inoculation sites. Flowers and leaf disks were surface sterilized by shaking the tissues in 1.2% sodium hypochlorite (20% Javex) plus 0.1% Tween 80 for 15 min, and washed three times in sterile distilled water. Sepals, petals, and pedicels were transferred aseptically from the surface-sterilized flowers to water agar medium (WA) and the surface-sterilized leaf disks were placed on paraquat-chloramphenicol agar (PCA) medium (26), all in 9-cm-diameter petri dishes. The tissues were incubated beneath cool-white fluorescent tubes (24-h photoperiod) at 20 to 22°C for 5 to 7 days, and subsequently examined with a dissecting microscope for conidiophores of *B. cinerea*.

Inoculum concentration and flower infection. Inflorescences each with 5 to 10 opened flowers were inoculated with *B. cinerea* in logarithmic increments from 10 to 10^6 conidia per ml of sterile distilled water plus surfactant, and with the water plus surfactant only. After the standardized incubation in high humidity and on the greenhouse bench, five flowers were detached from each of three replicate inflorescences per treatment and surface sterilized. Five sepals, five petals, eight stamens, the pistil, and the pedicel of each flower were used for estimation of sporulation incidence of *B. cinerea* in the respective organs.

Inoculum concentration and leaf infection. Leaves that were 4 to 6 weeks past the bud stage (buds 3 to 4 mm long) were inoculated with conidia at concentrations in logarithmic increments from 10 to 10^6 conidia per ml, and with petals that had been inoculated with the same concentrations of conidia. Checks were treated with sterile distilled water plus surfactant only. For each treatment, four leaves on each of three replicate plants were inoculated. Leaves treated with petal inoculum each received five petals. After inoculated plants were incubated in high humidity and on the greenhouse bench, five disks were cut from each leaf treated with conidia and one disk was cut from each site of petal adhesion on the leaves treated with petal inoculum, for a total of 20 disks for each replicate plant. Sporulation incidence of *B. cinerea* was estimated in disks of each treatment.

Flower age and infection. Freshly opened flowers of attached inflorescences were tagged daily for 7 days and inoculated with *B. cinerea* (10^5 conidia per ml of sterile distilled water plus surfactant) or with water plus surfactant only. After standardized incubation in high humidity and on the greenhouse bench, five flowers of each age group on each of three replicate inflorescences per treatment were detached and surface sterilized. Five sepals, five petals, eight stamens, the pistil, and the pedicel of each flower were used for estimating sporulation incidence of *B. cinerea* in the respective organs.

Leaf age and infection. Age of leaves was measured starting when the leaf bud was 3 to 4 mm long. These buds were tagged starting when the plants were 2 weeks old. Leaves were grouped according to age in weekly increments from 1 to 10 weeks and inoculated with conidia (10^6 conidia per ml) or with water plus surfactant. One-, 3-, 4-, 6-, 8-, and 10-week-old leaves were treated with petals that had been sprayed with the pathogen (10^5 conidia per ml) or with water plus surfactant. For leaves 3 to 10 weeks old, five petals were placed on each of three leaves of five replicate plants per treatment. For 1-week-old leaves (with laminae 12 to 20 mm wide) one petal was placed on each of six leaves of five replicate plants per treatment. In all instances, leaves of different age groups were inoculated simultaneously, and kept for the standardized periods in high humidity and on the greenhouse bench. Sporulation incidence of *B. cinerea* was estimated using five disks from each of 15 leaves per treatment, except in 1-week-old leaves in which one disk was cut from each of 30 leaves.

Experimental design and data analyses. A completely randomized design was used in all experiments, each of which was repeated once. Statistical computations were performed using the Statistical Analysis System (SAS Institute Inc., Cary, NC). Observations of repeated experiments were subjected to analyses of homogeneity of variance and the data were pooled when appropriate. All data were examined using analysis of variance (ANOVA) and the treatment means were compared using the Waller-Duncan Bayesian *k*-ratio *t* test (9,29). Regression analyses were used to determine relationships of inoculum concentration and of host age with incidence of sporulation. Linear, logistic, and monomolecular models were tested for goodness of fit of the regressions (8).

RESULTS

Effects of inoculum concentration in flowers. Sporulation incidence of *B. cinerea* in sepals, stamens, pistils, and pedicels increased from 0 to 94 to 98%, and in petals from 16 to 98% when inoculum concentration was increased from 10 to 10^5 conidia per ml (Fig. 1). The lowest concentration that resulted in sporulation of the pathogen was 10 conidia per ml for petals and 100 conidia per ml for other flower parts. Sporulation incidence in flower parts inoculated with 10^5 and 10^6 conidia per ml did not differ significantly ($P \leq 0.05$). A linear regression model adequately described the data obtained for $\log_{10}$ inoculum concentration (C) in relation to sporulation incidence (Y) in sepals, stamens, pistils, and pedicels, and a logistic regression model closely described the data for petals (Table 1). ANOVA and regression analysis (Table 1) indicated that C

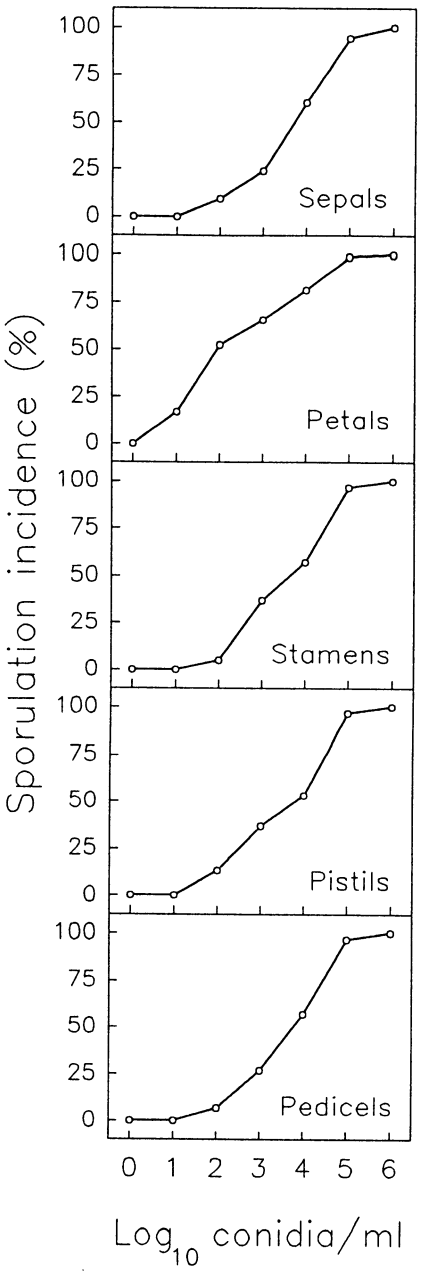

Fig. 1. Effects of inoculum concentration ($\log_{10}$ conidia per ml of sterile distilled water plus surfactant) of *Botrytis cinerea* on estimated incidence of sporulation of the pathogen in different parts of geranium flowers.

Table 1. Estimated parameter values and coefficients of determination (R^2) from regression models describing relationships between inoculum concentration ($\log_{10}$ conidia/ml) of *Botrytis cinerea* (C) and estimated (%) sporulation incidence(Y) of the pathogen in different parts of geranium flowers[a]

Flower part	Model[b]	Estimated parameters		R^2
		b_0	b_1[c]	
Sepal	Linear	−0.31	0.23	0.86
Petal	Logistic	−2.26	0.99	0.95
Stamen	Linear	−0.27	0.23	0.88
Pistil	Linear	−0.27	0.22	0.88
Pedicel	Linear	−0.32	0.23	0.86

[a] Data of two independent repetitions of the study did not differ significantly (F test) and were combined for analysis.

[b] The linear model is $Y = b_0 + b_1c + e_i$ and the logistic model is logit $Y = b_0 + b_1c + e_i$. The logistic transformation of Y is $\ln[(Y + k)/(1 - Y + k)]$ in which k is a constant ($0 < k < 1$). Unknown parameters b_0 and b_1 are each significant ($P \leq 0.0001$); e_i is the random variability of the models.

[c] All slopes differ significantly from zero ($P \leq 0.05$).

Table 2. Estimated concentration of inoculum of *Botrytis cinerea* required to produce 50% incidence of sporulation of the pathogen (EC_{50}) in different flower parts of geranium at 21°C

Flower part	EC_{50} (conidia/ml)[a]	Confidence interval (95%)
Sepal	3,325	3,020 < C < 3,726
Petal	815	733 < C < 890
Stamen	2,721	2,565 < C < 2,873
Pistil	3,162	2,683 < C < 3,675
Pedicel	3,675	3,311 < C < 4,160

[a] Values calculated from the regression models (Table 1).

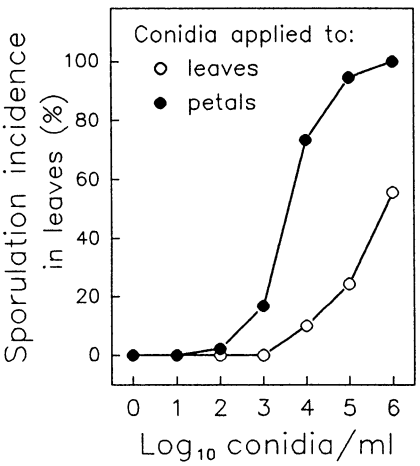

Fig. 2. Effects of conidium concentration of *Botrytis cinerea* on estimated incidence of sporulation of the pathogen in geranium leaves that were inoculated with conidia or with geranium petals that were sprayed with conidia 24 h earlier.

significantly affected Y and that the relationship was linear for all flower parts ($P \leq 0.05$). Coefficients of determination (R^2) obtained for the models were moderately high.

Regression models were used to estimate the inoculum concentration required to produce 50% incidence of sporulation (EC_{50}) in different flower parts. The EC_{50} value for petals was low (815 conidia per ml), but significantly higher (2,721 to 3,675 conidia per ml, $P \leq 0.05$) in the other flower parts (Table 2).

Effects of inoculum concentration in leaves. Sporulation incidence of *B. cinerea* in disks from leaves that had been inoculated with conidia was zero when the inoculum concentration was 10 to 10^3 conidia per ml, but increased as inoculum concentration was increased from 10^4 to 10^6 conidia per ml (Fig. 2). The highest sporulation incidence was 55%.

Sporulation incidence in disks from leaves treated with inoculated petals increased sigmoidally with $\log_{10}$ increments in inoculum concentration applied to the petals 24 h before they were positioned on the leaves (Fig. 2). Incidence on the disks was zero for an inoculum concentration of 0 and 10 conidia per ml, but increased from 2 to 100% as inoculum concentration increased from 100 to 10^6 conidia per ml. Relationships of conidia concentration and sporulation incidence in petals used as inoculum were similar to those described for petals in Figure 1.

Logistic regression models best described the data obtained for $\log_{10}$ inoculum concentration (C) in relation to sporulation incidence (Y) on leaves inoculated with conidia and on those treated with inoculated petals (Table 3). ANOVAs and regression analyses (Table 3) indicated that C significantly affected Y, that the relationship in leaves inoculated with conidia was significantly linear and squared, and that the relationship in leaves treated with petal inoculum was significantly linear. R^2 values for leaves inoculated with conidia and with petals were high and moderately high, respectively. Conidial concentration of *B. cinerea* required for 50% infection incidence of leaves, as estimated using the models, was about 200 times lower when the conidia were applied to petals that subsequently were used as inoculum, than when the conidia were applied directly to the leaves (Table 4).

Effects of flower age. Observations of experimental repetitions did not differ significantly ($P \leq 0.05$) and were pooled. Sporulation incidence of *B. cinerea* in the different parts of the geranium flowers increased with flower age (Fig. 3). Sporulation of the pathogen was observed on some petals, stamens, pistils, and pedicels, but not on sepals of flowers that were inoculated one day after opening, and on all parts of the flowers that were inoculated ≥2 days after opening. Sporulation incidence reached 95 to 100% in sepals, petals, stamens, pistils, and pedicels of flowers that were inoculated when 6, 4, 4, 5, and 6 or more days after opening, respectively.

The logistic regression model best fitted the data for sporulation incidence (Y) of *B. cinerea* in each of the different flower parts as a function of flower age (A). ANOVA and regression analysis (Table 5) indicated that the relationship between Y and A was significant and linear for pistils ($P \leq 0.0001$) and of the second order ($P \leq 0.0001$) for the other flower parts. The R^2 values were moderate to high.

The estimated age of flowers at which inoculation with 10^5 conidia of *B. cinerea* per ml resulted in 50% sporulation incidence of the pathogen in different flower parts ranged from 1.7 to 3.9 days after flower opening (Table 6). The EA_{50} values for petals, stamens, and pedicels were significantly lower than those for sepals and pistils.

Effects of leaf age. Sporulation incidence of *B. cinerea* in relation to leaf age followed a troughlike pattern when the leaves were inoculated with conidia or with inoculated petals, but, for each age group of leaves, was significantly higher ($P \leq 0.05$) when the petal inoculum was used (Fig. 4). For both inoculum types, sporulation incidence was high in newly emerged (1-week-old) leaves, declined as leaf age increased to 4 weeks, and increased when leaf age increased from 4 to 10 weeks. Sporulation incidence was significantly higher in 1- and 10-week-old leaves, and significantly lower in 3- and 4-week-old leaves, than in leaves of any other age ($P \leq 0.05$). The 10-week-old leaves were senescing.

A linear model best fitted the observed data for sporulation incidence (Y) as a function of leaf age (A) for both inoculum types of *B. cinerea* (Table 7). ANOVA and regression analysis indicated that A significantly affected Y and that the relationship was of the second order ($P \leq 0.0001$).

DISCUSSION

Observed effects of inoculum density and host age on sporulation incidence of *B. cinerea* in various flower parts and in leaves were indirect and presumably mediated by direct influence of these variables on infection and colonization of inoculated tissues by the pathogen. From observations we infer that conidia of *B. cinerea* can directly infect sepals, petals, stamens, pistils, and pedicels of geranium flowers. Conidia of the pathogen are also known to infect flower parts of pear (30), strawberry (5,20), and other hosts (18). Observations that conidia infected 1- to

Table 3. Estimated parameter values and coefficient of determination (R^2) from logistic regression models describing $\log_{10}$ concentration of conidia of *Botrytis cinerea* (C) applied to geranium leaves, and to geranium petals that after 24 h were positioned on leaves, in relation to sporulation incidence of the pathogen (Y) in inoculated leaves[a]

Inoculum applied to leaves[b]	Estimated parameters			
	b_0	b_1[c]	b_2	R^2
Conidia	−2.67	−0.37	0.14	0.96
Inoculated petals	−3.71	1.07	...	0.90

[a] Data of two independent repetitions of the study did not differ significantly (F test) and were combined for analysis.
[b] The models Logit $Y = b_0 + b_1c + b_2c^2 + e_i$ and Logit $Y = b_0 + b_1c + e_i$ are used for data of leaves inoculated with conidia and inoculated petals, respectively; b_0 to b_2 are estimated parameters each of which is significant ($P < 0.0001$), and e_i is the estimated variability associated with the models.
[c] All slopes differ significantly from zero ($P \leq 0.05$).

10-week-old leaves of geranium confirmed the considerations of Melchers (22) and others that the pathogen can infect the leaves directly. The PCA medium, which kills leaves and allows *B. cinerea* to sporulate rapidly (26), facilitated detection of the pathogen in symptomless leaves. *Botrytis cinerea* is possibly quiescent in geranium leaves while they are green, as it is in leaves of strawberry (4), potato (15), and raspberry (H. Yu and J. C. Sutton, unpublished observations).

Threshold concentrations of conidia required for *B. cinerea* to infect flower parts were extremely low (≤10/ml for petals and 10 to 100/ml for sepals, stamens, pistils, and pedicels), compared with the threshold value for 4- to 6-week-old leaves (10^3 to 10^4 conidia per ml) but increase in infection incidence with increased conidium concentration was generally more gradual in flower parts than in leaves. Thus, infection incidence in sepals, stamens, pistils, and pedicels increased linearly and with low slope values in response to logarithmic increments in conidium concentration (Table 1). Infection incidence of petals and leaves, however, increased logistically with $\log_{10}$ inoculum concentration. Wide variation in receptivity of each type of flower part to infection by *B. cinerea* could have contributed to the low increases in infection incidence. Variation in receptivity could be expected in view of age variation within populations of flower parts used in the studies, and the marked effects of age on infection incidence (Fig. 3).

Age, and by inference developmental stage, of flower parts and leaves were key factors affecting infection. From the estimates of sporulation incidence of the pathogen, receptivity of flower parts to infection was low immediately after flowers opened, but increased rapidly during the subsequent 3 to 5 days. Stamens were especially receptive to infection as indicated by the rates of increase in sporulation incidence and EA_{50} values (Tables 5 and 6). The trough-shaped patterns of sporulation incidence in leaves inoculated with conidia alone or with petals treated with conidia (Fig. 4) indicated that newly emerging leaves (1 week old) and mature

Table 4. Estimated concentration of conidia of *Botrytis cinerea* required to produce 50% sporulation incidence of the pathogen (EC_{50}) in 1-cm-diameter disks cut from leaves inoculated with the conidia or with petals inoculated 24 h earlier with the conidia

Inoculum applied to leaves	EC_{50} (conidia/ml)[a]	Confidence interval (95%)
Conidia	2,225,394	2,013,675 < C < 2,435,336
Inoculated petals	11,184	10,458 < C < 12,056

[a] Values calculated from the regression models (Table 3).

to senescent leaves (8 to 10 weeks old) were highly receptive to infection, and that receptivity of expanding leaves (4 weeks old) was low. Receptivity of strawberry leaves to infection by *B. cinerea* was also found to be high when the leaves emerged and low when the leaves were fully expanded, but receptivity remained low when the leaves aged unless the tissues were surface sterilized before inoculation (4).

Nutrient availability and tissue receptivity were probably important factors contributing to the observed patterns of infection incidence in the flower parts and leaves. Simple carbon and nitrogen sources as well as other nutrients in host exudates and pollen diffusates stimulate conidium germination, hyphal growth, and appressorium formation by *B. cinerea* in moisture on host surfaces, and increase penetration of hosts by the pathogen (2,3, 16,18). Exuded nutrients can serve as energy sources of *B. cinerea* and induce production by the pathogen of enzymes that macerate host tissues (2). Exudate concentration and pollen density can be much higher on flowers than on leaves (18,28), a factor that could have contributed to the low thresholds of conidium concentration required to infect the flowers compared with the leaves of geranium. Increased exudation and pollen release from flowers and increasing tissue receptivity were possible factors contributing to the marked increases in infection incidence of geranium flower parts as flowers increased in age. Physiological changes such as increased permeability of cell membranes

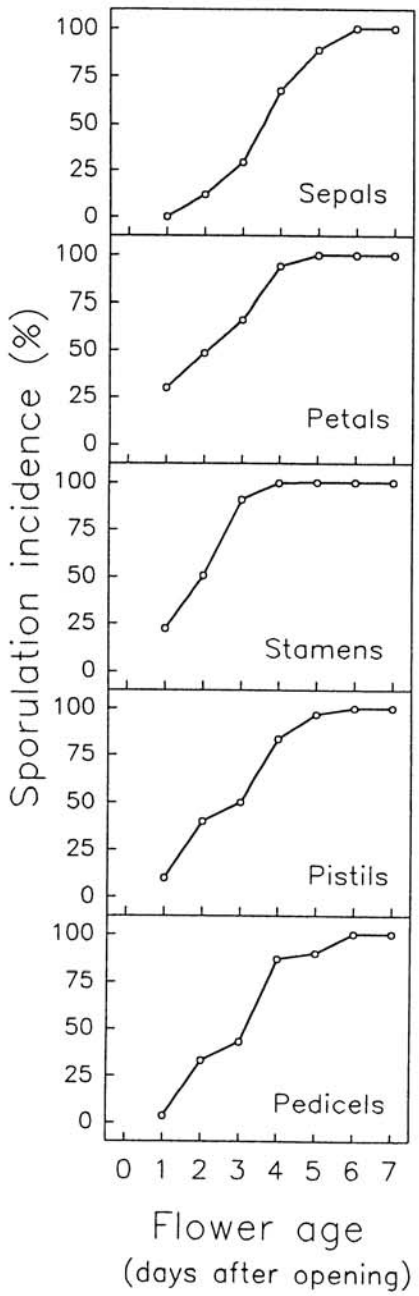

Fig. 3. Effects of flower age on estimated incidence of sporulation of *Botrytis cinerea* in different parts of geranium flowers inoculated with a conidial suspension (10^5 spores/ml) of the pathogen.

and higher sugar levels may increase receptivity of flower tissues as they age (10, 19). In geranium leaves, changes in cuticle thickness, trichome densities, exudate levels, pollen deposition, and tissue physiology were potential factors contributing to the trough-shaped patterns of infection incidence in relation to leaf age. Cuticular waxes, for example, can be thin in young and old leaves but thick in middle-aged leaves (25), a pattern consistent with the observed receptivity of the leaves to infection by *B. cinerea*. Inoculated petals that were used as inoculum on leaves provided a substrate and food base for the pathogen (18) and increased infection efficiency of

conidia by 200-fold or more (Table 4). Microflora on inoculated geranium tissues was probably sparse, given that RH in the greenhouse only rarely exceeded 80%, and thus probably did not markedly affect infection by *B. cinerea*.

Relationships of inoculum concentration and host age to inferred infection incidence by *B. cinerea* have substantial implications in epidemics of gray mold in geranium production systems. For example, a density of initial inoculum (conidia) that is below the threshold for leaf infection but above that for flower infection could delay epidemics until after flowering begins. In this situation, production of subsequent inoculum on the flowers could raise inoculum levels sufficiently that the leaves become infected. The marked reduction in threshold concentration of conidia for *B. cinerea* to infect leaves when petal inoculum was used (10 to 100 conidia per ml) further underscored the potential for accelerated disease progress on the foliage after the flowers develop and petals fall (11,22). Should levels of initial inoculum be high, however, epidemics could potentially be initiated on the leaves before flowering begins. The gradual increase in infection incidence of flower parts in response to increased inoculum density would be expected to retard disease progress.

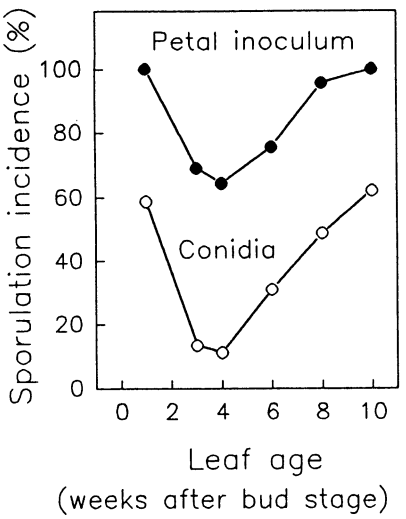

Fig. 4. Effects of age of geranium leaves after the bud stage when inoculated with conidia (10^6/ml) of *Botrytis cinerea* or with geranium petals inoculated 24 h earlier with conidia (10^5/ml), on sporulation incidence of the pathogen in the leaves.

The quantitative observations of inferred infection have applications in the development and implementation of methods to control gray mold. The data provide a basis for rational selection of conidium concentration and host tissue when fungicides, biological control agents, microclimatic regulation, and other disease control methods are evaluated. They also could be used in conjunction with other epidemiological information (e.g., 12,13,14) for development of disease forecasting or warning systems. Improved control measures optimally timed by warning systems could increase the efficiency of gray mold management and help to reduce fungicide dependency in geranium production systems.

ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada (grant OGP0006119 to the second author).

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Table 5. Estimated parameter values and coefficient of determination (R^2) from logistic regression models[a] describing the effect of flower age (A) when inoculated with *Botrytis cinerea* on sporulation incidence (Y) of the pathogen in different parts of geranium flowers[b]

	Estimated parameters			
Flower part	b_0	b_1[c]	b_2	R^2
Sepal	−5.04	1.73	−0.07	0.95
Petal	−2.67	1.64	−0.11	0.93
Stamen	−9.00	6.65	−0.50	0.81
Pistil	−1.50	0.54	...	0.71
Pedicel	−2.93	1.35	−0.10	0.82

[a] The model used for sepals, petals, stamens, and pedicels was Logit $Y = b_0 + b_1A + b_2A^2 + e_i$ and the model for pistils was Logit $Y = b_0 + b_1A + e_i$. The logistic transformation of Y was $\ln[(Y + k)/(1 - Y + k)]$ in which k was a constant, b_0 to b_2 were unknown parameters, each of which was significant ($P \leq 0.0001$) and e_i was random variability of the models.

[b] Data of two independent repetitions of the study did not differ significantly (F test) and were combined for analysis.

[c] All slopes differed significantly from zero ($P = 0.05$)

Table 6. Estimated age of geranium flowers when inoculated with *Botrytis cinerea* (10^5 conidia/ml) that resulted in 50% sporulation incidence of the pathogen (EA_{50}) in different parts of inoculated flowers at 21°C

Flower part	EA_{50} (days)[a]	**Confidence interval (95%)**
Sepal	3.9	3.8 < A < 4.0
Petal	2.4	2.3 < A < 2.8
Stamen	1.7	0.7 < A < 1.9
Pistil	3.9	3.8 < A < 4.0
Pedicel	2.7	2.6 < A < 2.8

[a] Days after flowers opened. Values calculated from the regression models (Table 5).

Table 7. Estimated parameter values and coefficient of determination (R^2) from a linear regression model[a] describing effect of age of geranium leaves (A) when inoculated with conidia of *Botrytis cinerea* (10^6/ml) or with geranium petals inoculated with conidia (10^5/ml), on sporulation incidence (Y) of the pathogen in inoculated leaves[b]

	Estimated parameters			
Inoculum applied to leaves	b_0	b_1[c]	b_2	R^2
Conidia	0.66	−1.91	0.02	0.70
Inoculated petals	1.06	0.14	0.01	0.69

[a] The linear model $Y = b_0 + b_1A + b_2A^2 + e_i$ was used for leaves inoculated with conidia and those inoculated with petals. The unknown parameters b_0, b_1, and b_2 were significant ($P \leq 0.0001$), and e_i was the random variability of the model.

[b] Data of two independent repetitions of the study did not differ significantly (F test) and were combined for analysis.

[c] All slopes differed significantly from zero ($P = 0.05$)

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